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(54) Title: NOVEL THERMOPHILIC POLYMERASE III HOLOENZYME

(57) Abstract: The present invention relates to gene and amino acid sequences encoding DNA polymerase III holoenzyme subunits and structural genes from thermophilic organisms. In particular, the present invention provides DNA polymerase III holoenzyme subunits and accessory proteins of *T. thermophilus*. The present invention also provides antibodies, primers, probes, and other reagents useful to identify DNA polymerase III molecules.

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FIGs. 9A and B. SDS-PAGE analysis of the fraction from the  $\text{Ni}^{++}$ -NTA column purification of N-terminal tagged *T. thermophilus* DnaX.

FIGs. 10A and B. SDS-PAGE analysis of the fraction from the avidin column purification of N-terminal tagged *T. thermophilus* DnaX.

5 FIG. 11. Western analysis of various antiserum dilutions for determination of dilutions to use in *T. thermophilus* DnaX detection.

FIG. 12. Western analysis of various *T. thermophilus* DnaX dilutions for determination of the limit of DnaX detection at antiserum dilution of 1:6400.

10 FIG. 13 The DNA sequence (SEQ ID NO:9) of the *T. thermophilus* *holA* gene ( $\delta$  subunit).

FIG. 14. The amino acid sequence (SEQ ID NO: 10) of *T. thermophilus*  $\delta$ -subunit (*holA* gene).

15 FIG. 15. Alignment of the amino acid sequence of  $\delta$  from *T. thermophilus* and *E. coli*.

FIG. 16 Alignment of the amino acid sequence of  $\delta$ -subunit from *A. aerolicus*, *T. thermophilus*, *B. subtilis*, *E. coli* and *H. influenzae*.

FIG. 17 Biotin blot analysis of growth/induction time optimization of expression of *T. thermophilus*  $\delta$  by pA1-NB-TD/AP1.L1.

20 FIG. 18. Optimization of precipitation of *T. thermophilus*  $\delta$  by ammonium sulfate.

FIGs. 19A and B. SDS-PAGE analysis of fractions from the  $\text{Ni}^{++}$ -NTA column purification of *T. thermophilus*  $\delta$ .

25 FIG. 20. Protein concentration profile of fractions from the avidin column purification of *T. thermophilus*  $\delta$ .

FIG. 21 SDS-PAGE analysis of fractions from the avidin column purification of *T. thermophilus*  $\delta$ .

FIG. 22. The DNA sequence (SEQ ID NO: 16 ) of the *T. thermophilus* *holB* gene encoding the  $\delta'$ -subunit of the *T. thermophilus* Pol III holoenzyme.

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## SEQ ID NO:9

atgggtcatcgcccttcaccggggatcccttctctggcgcgaggccctcttagaggaggca  
aggcttaggggctttcccgcttcacggagccacccggaggccctggcccaggccctc  
gccccggggttttcggggggcgggggggcgatgctggacctgagggaggtgggggaggcg  
gagtggaaaggccctaaagccctcctggaaagcgtgcccggggcggtccccgtctctctc  
ctggaccctaagccaagccctcccgggcgccctctaccggaaccgggaaaggcgggac  
tccccacccccaaagggaaggacctggtgcggcacctggaaaaccgggccaaggcgctg  
gggctcaggctcccgggcggggtggcccagtagctggcctccctggagggggacctcgag  
gcccgggaacgggagctggagaagcttgccctcctctccccctccccctcaccctggagaag  
gtggagaagggtggtggccctgaggccccccctcacgggctttgacctggtgcgctccgtc  
ctggagaaggacccccaggaggccctcctggcctcaggcgccctcaaggaggagggggag  
gagccctcaggctcctcggggcccctctcctggcagttcgccctcctcgcccgggccctc  
ttctcctccgggaaaaacccaggcccaaggaggaggacctcgcccgccctcgaggccac  
ccctacgcgcgcaaaaaggccctggaggcggcgaggcgcccttacgggaagaagccctcaag  
gaggccctggacgccctcatggaggcggaagaggcccaagggggggaaagaccatgg  
cttgccctggaggcggcgtcctccgcctcgcccggtga

FIG. 13

The DNA coding sequence of the *T. thermophilus hola* gene (SEQ ID NO:9).

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SEQ ID NO:10

MVIAFTGDPFLAREALLEEARLRGLSRPTEPTPEALAQALAPGLFGGGGAMLDLREVGEA  
EWKALKPLLESVPEGVPVLLLDPKPSPSRAAFYRNRRERDFTPKGKDLVRHLENRAKRL  
GLRLPGGVAQYLASLEGDLALERELEKIALLSPLTLEKVEKVVALRPPLTGFDLVRVS  
LEKDPKEALLRLRLKEEGEEPLRLLGALSWQFALLARAFFILRENPRPKKEEDLARLEAH  
PYAAKKALEAARRLTEEALKEALDALMEAKRAKGGKDPWLALAEAAVLRIRAR

FIG. 14

The amino acid sequence of *T. thermophilus*  $\delta$ -subunit.

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